Chemical Engineering Journal 255 (2014) 171–177

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

A new approach for estimating aerobic–anaerobic biofilm structure in wastewater treatment via dissolved oxygen microdistribution

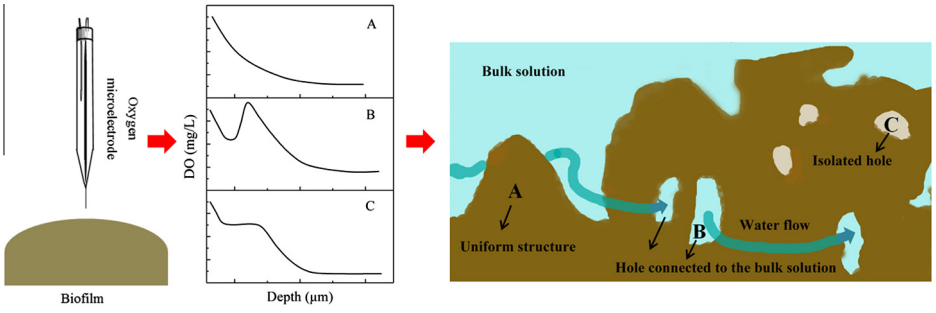
Yun-Fang Ning [a], You-Peng Chen [b,*], Yu Shen [b], Ni Zeng [a], Shao-Yang Liu [c], Jin-Song Guo [a,b], Fang Fang [a,*]

[a] Key Laboratory of Three Gorges Reservoir Region's Eco-Environments of MOE, Chongqing University, Chongqing 400045, China
[b] Key Laboratory of Reservoir Aquatic Environment, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China
[c] Department of Chemistry and Physics, Troy University, Troy, AL 36082, USA

HIGHLIGHTS

- The DO microdistributions in a biofilm were measured.
- The thicknesses of aerobic and anaerobic layers of a biofilm were determined.
- A method for estimating biofilm structure via DO microdistribution was presented.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form 6 June 2014
Accepted 10 June 2014
Available online 18 June 2014

Keywords:
Dissolved oxygen
Aerobic layer thickness
Anaerobic layer thickness
Biofilm structure
Microelectrode

ABSTRACT

This study proposed a new method for determining the thicknesses of aerobic and anaerobic layers in a biofilm. The transport of dissolved oxygen (DO) or nutrients from the bulk solution to the biofilm was more closely related to the thicknesses of the aerobic and anaerobic layers than to the biofilm structure. The DO microdistribution of the biofilm from a sequencing batch biofilm reactor (SBBR) was measured using microelectrode. The measurements were used to speculate the biofilm structure. The average and maximum thicknesses were 365 and 640 μm in the test biofilm, respectively. The mean thicknesses of the aerobic and anaerobic layers were 270 and 240 μm, respectively. The maximum thickness of the aerobic layer was 420 μm. Aerobic oxidation mainly occurred at the top half of the biofilm during the SBBR aeration stage. The biofilm was heterogeneous. However, many holes were detected inside the biofilm. Some of these holes were connected to the bulk solution and allowed the bulk solution to enter and exit freely, whereas the others were isolated.

1. Introduction

Compared with the activated sludge process, the biofilm technology has smaller layout area, more microbial populations, stronger impact resistance, and more stable ecological system in wastewater treatment [1,2]. In addition, biofilms have higher efficiency and wider application in nitrogen removal systems than activated sludge [3,4]. In biological nitrogen removal, the traditional nitrification and denitrification or the innovative ones (e.g., SHARON [5], ANAMMOX [6], CANON [7,8]) follow two principal steps. One is ammonia oxidation, and the other is the conversion of oxidation products (and ammonia) to nitrogen gas, which can be simultaneously achieved in the aerobic and anaerobic layers inside the biofilm [9,10]. Layered and orderly ammonia oxidation and nitrogen gas generation are crucial to the efficient removal of nitrogen from wastewater [11].

* Corresponding authors. Tel.: +86 23 65935818 (Y.-P. Chen), +86 23 65127370 (F. Fang).
E-mail addresses: ypchen@cigit.ac.cn (Y.-P. Chen), fangfangcq@cqu.edu.cn (F. Fang).

http://dx.doi.org/10.1016/j.cej.2014.06.042
1385-8947/

In the biofilm technology, the transfer and consumption of dissolved oxygen (DO) serves important functions in nitrogen removal. Excessively high DO transfer resistance in the biofilm makes the aerobic layer too thin and complicates ammonia oxidation. Conversely, excessively low DO transfer resistance makes the anaerobic layer too thin and slows down denitrification. DO level also significantly affects nitrogen removal efficiency. The DO concentration and appropriate thicknesses of the aerobic and anaerobic layers in the biofilm are important factors affecting the coupling of the two steps in nitrogen removal [10,12]. Therefore, determining the thicknesses of the aerobic and anaerobic layers individually is important, not just the total thickness of the biofilm. However, a convenient and fast method for determining the thicknesses of the aerobic and anaerobic layers is lacking.

The transport of DO and nutrients from the bulk solution to the inner part of the biofilm is more closely related to the thicknesses of the aerobic and anaerobic layers [12] than to the biofilm structure [13–16]. The general parameters used to describe biofilm structure include roughness coefficient, surface area coverage, biovolume, and thicknesses (average and maximum) [17–19]. These parameters are 1D, providing information only in the direction perpendicular to the biofilm surface. To describe biofilm structure in 3D, confocal laser scanning microscopy (CLSM) has been applied in many studies [19–22]. Aside from CLSM, electrochemical analysis with microelectrodes can be used as a new approach for the 3D *in situ* investigation of biofilm internal structure.

This study determined the mean and maximum thicknesses of the aerobic and anaerobic layers of the biofilm by using microelectrodes. The DO microdistribution inside the biofilm was also measured and used to speculate the biofilm structure.

2. Materials and methods

2.1. Biofilm and analytical methods

The biofilm tested in the current study was sampled from a 20 L sequencing batch biofilm reactor (SBBR) with aeration and nonaeration durations of 2 and 2 h, respectively. The biofilm was attached to flat and rough gravels in the SBBR, and the wastewater was purified by the CANON process. The components of the inorganic synthetic wastewater used as influent are listed in Table 1. NH_4^+–N, NO_3^-–N, NO_2^-–N, and TN were measured according to standard methods [23]. A portable digital DO meter (YSI, Professional ODO™, YSI Co., USA) was used to determine the DO concentration in the SBBR.

Table 1
Influent inorganic synthetic wastewater in SBBR.

Inorganic synthetic wastewater	NH_4^+–N	TP	pH	Nutrient solution
Concentration (mg/L)	200	20	7.8–8.2	2 (mL/L)

2.2. In situ measurements

The SBBR was under aerated state and the DO concentration in the bulk solution was controlled at 2.0 mg/L when the biofilm was measured by a DO microelectrode. The oxygen micromeasurement system consisted of a 3D microelectrode propeller whose maximum accuracy was 10 μm and an oxygen microelectrode (Unisense, OX10, Denmark) whose tip diameter was 10 μm and response time was approximately 0.4 s. The microelectrode was connected to a picoampere meter (Unisense, MM336155, Denmark), and the measuring signals were recorded on a PC with a custom-made data acquisition system.

A schematic of the *in situ* DO measurement system is shown in Fig. 1. The system consisted of high-pressure nitrogen and oxygen cylinders, two flow meters, a gas mixing chamber, a jar with rubber plug, a peristaltic pump, an aerator pipe, three magnetic stirrers, a special measurement vessel, an oxygen meter (YSI), and an oxygen microsensor. The special measurement vessel was made with polymethyl methacrylate. The length, width, and height of the vessel were 20, 10, and 6 cm, respectively. Wastewater in the jar with controlled DO level was pumped to the special measurement vessel through an external circulation channel.

2.3. Biofilm measurement

A lathy glass tube (tip diameter: 20 μm) was used to measure the thickness of the biofilm. The glass tube and biofilm were fixed on the 3D microelectrode propeller and measurement vessel, respectively. A schematic of the thickness determination apparatus is shown in Fig. S1. The measurement vessel was parallel to the ground before the measurement started. During the measurement, the tip of the lathy glass tube approached the gravel surface until the tip touched the surface. This phenomenon was observed with a binocular magnifier at 5× magnification. The reading on the 3D microelectrode propeller was recorded as X_0. The lathy glass tube

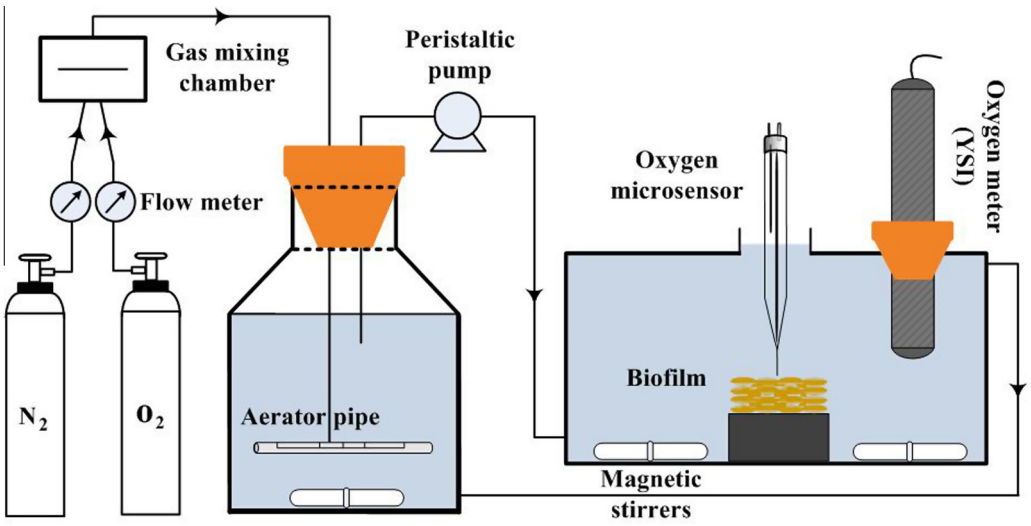


Fig. 1. Schematic of the *in situ* system for measuring DO concentration in a biofilm.